BACILLUS MORULANS, N. SP

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A BACTERIAL ORGANISM FOUND ASSOCIATED WITH CURLY TOP OF THE SUGAR BEET

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WITH SEVEN FIGURES IN THE TEXT

The writer, in conjunction with Prof. Ralph E. Smith, has been investigating a peculiar and fundamentally important plant disease, the so-called curly top of the sugar beet and related plants. Two joint publications[1] have already been made upon this subject in which there has been briefly announced the discovery of a bacterial organism found constantly associated with the disease in the tissues of affected plants. It has been stated further that, although obtained so regularly and abundantly in cultures

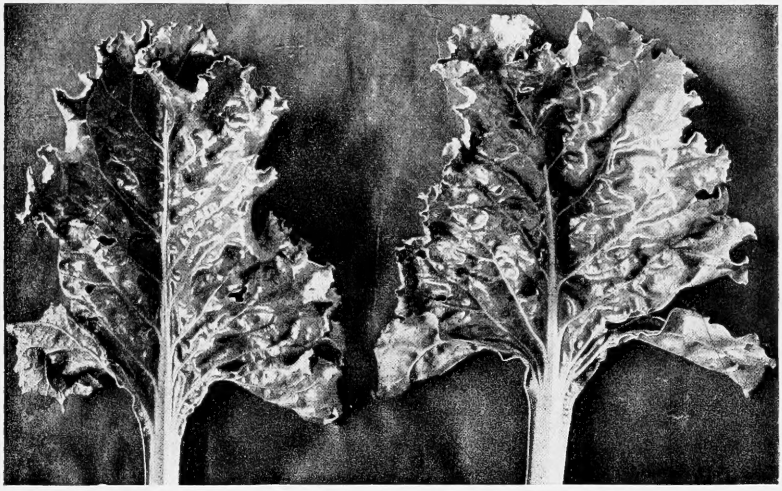

FIG. 1. WRINKLED TYPE OF BEET LEAVES

Not curly top, but having in the sieve tubes occasional groups of the bacterial bodies found in great abundance in curly top beets.

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[1] Smith, R. E., and Boncquet, P. A. New light on curly top of the sugar beet. Phytopath. **5**: 103–107. 1915.

——— Connection of a bacterial organism with curly leaf of the sugar beet. Phytopath. **5**: 335–342. 1915.

FIG. 2. SUGAR BEET PLANT TYPICALLY AFFECTED WITH CURLY TOP

from diseased plants or portions of plants, it has not been possible to produce curly top by inoculation with cultures of this organism; that the same organism has been isolated from the surface of beet seed, the surface of normal sugar beet leaves and from the soil about the roots of sugar beets. Also that certain bodies which seem to represent the same organism have been found in great abundance in curly top sugar beets, in the interior of the sieve tubes, accompanying a specific lesion in the phloem, and that similar bodies, in varying but much less abundance, were found in the same tissue in supposedly normal beets or those with various morphological irregularities of the foliage (fig. 1). Whatever may be the entire significance of the organism in question, its peculiarly abundant occurrence in connection with the sugar beet and its apparent relation to curly top have seemed to justify its careful study, and it is the purpose of the present article to describe more in detail the characteristics of this species, to which the name *Bacillus morulans* has been given.

THE DISEASE

The disease of sugar beets called curly top is of annual occurrence throughout the sugar beet growing regions of Colorado, Utah, Idaho and California. The severity of the disease, however, varies greatly from year to year. Some years veritable disasters are produced by curly top, thousands of acres of sugar beets being totally destroyed after all the expense of preparing the ground and planting the crop has been undergone.

Symptoms on leaves

The comparative size of the inner and outer leaves is altered. The inner leaves are dwarfed, the petiole especially becoming shorter and flatter than the normal, while the outer leaves, if already full grown before the disease becomes apparent, maintain their natural size and shape and, for some time at least, their color, although they may finally turn yellow and die prematurely. The first symptom of abnormality plainly visible to the eye is a distinct transparency of the finest venations of the youngest leaves. This transparency starts at the base of the leaf blade. Gradually the abnormality works higher on the leaf until finally the whole leaf is affected. The youngest leaves are first to suffer; the older ones (such as are not already full grown) show the symptoms as their expansion and growth progress. Almost simultaneously with the appearance of the transparency of the veins small warty protuberances appear upon the veins on the under surface of the leaves, eventually even upon those which are of the smallest size visible to the eye (fig. 2). The margins of the affected leaves then begin to curl slightly upward so as to expose the lower

surface. As these symptoms gradually increase, working outward from the innermost to the outer leaves, black spots soon appear in the principal leaf venation and especially in the youngest petioles. These black spots often break out through the cortical parenchyma and epidermis, allowing a black syrupy substance to ooze out, which upon drying produces in severe cases a crusty appearance upon the petioles. The interrupted and irregular expansion of the leaf venation soon brings about a very characteristic leaf curl. This curling for the most part is not parallel with the main midrib but extends more or less along the margin of the leaf and converges at the apex. If the plants are badly affected when very young they may be entirely killed; otherwise when the disease is advanced the outer leaves become yellow and die while the youngest may remain for a long time practically unchanged or occasionally may later resume

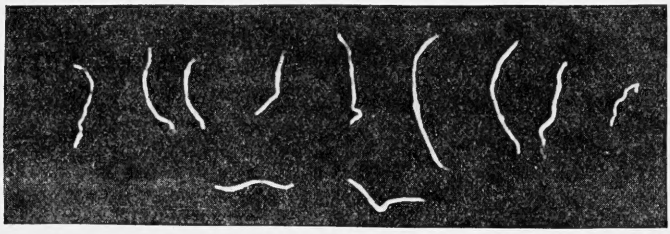

FIG. 3. SUGAR BEET ROOT TIPS, SHOWING CHARACTERISTIC BENDING AND SWELLING

growth and become apparently free from the above-mentioned symptoms. The latter represents a condition of recovery from the disease. In such cases the young leaves are also dwarfed but the venation is normal, the warty protuberances are absent and the leaf does not curl. In this condition, however, the whole leaf is of a denser green color, harder and thicker. The beet seems to have undergone a struggle similar to that which mesophytic plants undergo when they are transplanted to a desert habitat. In such cases the roots may develop to normal size even when the tops are much suppressed.

Symptoms on roots

It is only when the leaves have been thoroughly affected that the roots begin to show abnormality. This is characterized by the multiplication of rootlets. The older ones begin to die while at their base new ones push forth. This continuous destruction of the rootlets brings about a warty appearance, the bases of the masses of rootlets protruding slightly from the surface of the beet. On careful examination it will be observed that each rootlet, after it reaches an appreciable length and before it dies, has

several abnormal bendings; the angle of each bend is slightly swollen and if the rootlet is far advanced the swollen region appears to be necrotic (fig. 3). When the main root is cut transversely the successive rings of vascular tissue appear discolored. On careful examination it will be observed that the phloem is the only part of the vascular system which suffers severely. This phloem discoloration is observed more or less throughout the whole system in the veins as well as in the roots. This, however, only becomes apparent to the naked eye when the disease has reached its severest aspect.

Cause of the disease

It was E. D. Ball[2] who discovered that the sting of the insect *Eutettix tenella* Baker is a necessary factor in the causation of this disease. His observations were confirmed by Shaw,[3] and very fully tested and confirmed by Smith and Boncquet. The latter, however, together with Hartung,[4] proved a fact which had previously been suspected, that *Eutettix tenella* is not the fundamental factor in the causation of this disease, but rather must be a carrier of a second factor, presumably a parasitic micro-organism. This discovery made very important a thorough search for and study of all micro-organisms which possibly could be found in connection with the disease, and it is with this portion of the study of curly leaf that the present article has to do.

BACTERIOLOGICAL INVESTIGATIONS

The methods and detailed results through which the conclusion was reached by the writer that *Bacillus morulans* inhabits constantly and specifically plants affected with curly top, as well as occurring in certain other situations may first be described. It was decided at the outset to make a very thorough and accurate search for any organism which might be present in the tissues of plants affected with the disease. The unfavorable or at best uncertain results reported by previous investigators along this line led to the belief that the problem would be a difficult one,

[2] Ball, E. D. The beet leaf hopper. Utah Agr. Exp. Sta. Ann. Rept. **16**: 16. 1905.

——— The Genus Eutettix. Proc. Davenport Acad. Sci., **12**: 41 and 84. 1907.

——— The leaf hoppers of the sugar beet and their relation to the "curly leaf" conditions. U. S. Dept. Agr. Bur. Ent. Bul. 66, pt. 4. 1909.

[3] Shaw, H. B. The curly top of beets. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 181. 1910.

[4] Boncquet, P. A. and Hartung, W. J. The comparative effect upon sugar beets of Eutettix tenella Baker from wild plants and from curly leaf beets. Phytopath. **5**: 348–349. 1915.

but at the same time a study of the nature of the disease had led to a very strong feeling that some parasitic micro-organism, of which the insect *Eutettix tenella* was presumably a carrier or secondary host, must be involved in this disturbance. Assuming then that the sought-for organism would be an obscure one and difficult to demonstrate by ordinary cultural or histological methods, various special and somewhat elaborate culture methods were attempted.

Preparation of media

The following media which seemed most promising for this purpose were prepared:

Filtered beet juice. For this purpose the plants from which the juice was desired were washed as thoroughly as possible in sterilized water and then ground fine in a meat grinder. In most cases 100 cc. of distilled water was added to each 100 grams of beet pulp and the mass was then covered and left standing for two hours. The crushed material was subsequently put into clean cheesecloth and the juice squeezed out in a press. The juice thus obtained was subsequently diluted twice its volume with n/6 salt solution. Various degrees of dilution have been employed, however, from the original juice up to about ten to one, either in salt solution or water. In some cases the solution was then titrated and brought to the neutral point of phenolphthalein with sodium hydroxid. This juice was now clear, slightly brown and passed easily through a common filter paper. After it had been filtered through paper it was passed through a medium-dense Berkefeld filter candle for purposes of sterilization. An apparatus was especially devised for this purpose, a form of which is described in another article in the present number of Phytopathology. In order to be sure that no contamination had occurred during the manipulation, the tubes after filling were kept in the incubator for two days at 30°C. It is believed that this apparatus and method is worthy of considerable employment in the preparation of culture media for use in plant pathology.

Aseptic, unheated beet slices. These were prepared in the following manner: Sound, healthy beets were selected, thoroughly cleaned and immersed in boiling water for three minutes, in this way sterilizing the surface but not heating or changing in any manner the tissues deeper in the beet. They were then cut into slices with a carefully sterilized knife. Each slice was then put into a sterile petri dish into which previously ordinary nutrient agar had been poured.

Beet broth. Three hundred grams of beet leaves were cut into small pieces and boiled for an hour in 0.5 litre of water. Water was then added to make up to 1 litre and left standing for two hours. It was then filtered through cotton and 500 cc. of this beet extract added to 1 litre of Liebig's broth. The Liebig's broth had previously been prepared in the following way: 2 grams of Liebig's extract, 10 grams of Witte's peptone, and 5 grams of sodium chlorid were added to 1 litre of water. This medium was subsequenty neutralized to phenolphthalein with sodium hydroxid and after addition of the beet juice was brought up to 0.5 per cent acidity with malic acid. The same medium was also prepared with an increased proportion of beet extract.

Artificial media. A protein- and peptone-free medium was composed with the supposition that the organism did not attack the higher nitrogen compounds of the beet. Therefore several of the amino acids were used as the nitrogen supply. Alanin, leucin and tyrosin were used. Asparagin, although not an amino acid was also considered a possible favorable source of nitrogen for the parasitic organism. All these compounds were used in a dilution of 0.5 gram to 1 litre of water. The necessary minerals were added in the following form and proportion:

Magnesium sulfate	0.2 gram
Ammonium phosphate	0.5 gram
Potassium nitrate	0.2 gram
Calcium hydroxid	5 cc. of a saturated solution
Ferric chlorid	trace

These artificial media were sterilized in the Arnold sterilizer for fifteen minutes upon three consecutive days. Special glycerin and glucose media were also prepared. For this purpose 1 per cent glycerin was added to a part of the asparagin medium. So also 5 per cent glucose was added to another portion. The glucose medium was especially used for anaerobic purposes.

Other media. Ordinary media such as nutrient bouillon, potato glucose bouillon, bean pods, milk, litmus whey, nutrient agar and nutrient gelatin were prepared according to the standard methods.

Methods attempted for separating parasites from the plant

In order to separate the assumed parasites from the plant and obtain them in pure culture the following technique was used:

Surface-disinfected plant parts placed in culture medium. The usual method employed in this sort of work consists in soaking the tissue to be employed for a given length of time in mercuric chlorid and then washing off the same with sterilized water before placing the tissue in the culture medium. A need of standardizing this method was felt, inasmuch as there is no assurance, as it is usually described, whether on the one hand the disinfection was sufficiently thorough to kill all surface organisms or whether on the other hand the material was washed sufficiently to remove all the mercury and prevent its being carried over into the culture medium. The method consists in dry sterilizing a number of cotton-plugged flasks of 50 cc. capacity or any other desired size. At the same time larger flasks, likewise cotton-plugged and filled with distilled water, are made sterile in the autoclave. Other requisites are supplies of 95 per cent alcohol and 1 to 1000 solution of mercuric chlorid in water. The material from which cultures are desired, after thorough wiping with cotton swabs in 95 per cent or absolute alcohol in a photographic tray, is cut into convenient sized fragments, but no smaller than necessary. These are placed in one of the empty sterilized flasks and covered for a moment with the alcohol for the purpose of removing air bubbles. The alcohol is immediately poured off again and the flask nearly filled with mercury

solution so that all the material will be submerged. This is allowed to remain for the desired length of time, depending upon the nature of the tissue. The petioles and main veins of sugar beet leaves, especially fairly old leaves, will usually stand twenty minutes, but with leaf blades and other more delicate material ten minutes has been found the maximum time which can be used without too severe burning. Cut surfaces will naturally absorb more of the solution than those protected by the natural covering of the plant, and this can be taken into account both in consideration of the length of time which the tissue will stand without being burned by the mercury and also the time necessary for washing it out again. On this account it is best to cut the tissues as little as possible before disinfecting. After the desired time has elapsed a piece of brass wire gauze, bent to form a cap over the mouth of the flask, is sterilized in the flame, placed in position and the mercury poured off. The flask is then filled with sterilized water from the large flask and the water of this first washing, after having the material well shaken up in it, is poured off immediately and more water poured in. The process of pouring off and refilling is then continued at gradually increased intervals; the length of each must depend upon the nature of the material. It was found, however, that if the amount of material in the flask is comparatively small in proportion to its capacity, which should always be the case, six changes of water, extending over a period of two hours, is amply sufficient in every case. In this case the first five changes can be made during the first hour and the last one at the end of the second hour. If one wishes to practice extreme caution the mouth of the flask may be flamed and the cotton stopper replaced after each change of water, but this has not been found necessary so long as the amount of water is sufficient to thoroughly submerge all the material. In our work the wire screen is usually left over the mouth of the flask and this is freshly flamed before each change of water. After the process is completed the material is taken out of the last water with flamed forceps, broken into small pieces if necessary and thoroughly crushed with the same instruments and dropped into the culture liquid.

Piece cultures. It was thought that a gradual adaptation from the plant in which the organism is living to the medium in which it was attempted to grow it might be necessary to insure success; therefore the diseased tissue was so transferred as to disturb as little as possible the cells of the beet. For this purpose glass tubes were drawn out to 2 mm. diameter. After sterilizing by heat they were aseptically inserted into the diseased regions of the beet to a depth of 1 cm. The tube was then withdrawn, bringing with it a portion of the beet tissue and the terminal part containing the tissue was carefully broken off with sterilized forceps

and dropped into the medium. In this way both ends of the tissue slightly protruded from the glass tube and came into direct contact with the culture medium. The slow diffusion of the latter was supposed to secure a gradual change of habitat in such a way as not to hinder too severely the growth of the parasite. Tissue was thus removed from the petiole, from inside the root and from the larger veins of the leaf, after surface sterilization with a flame or boiling water, afterwards cutting into the interior with a sterilized knife and then introducing the glass tube to take out a small core of tissue.

Results of isolation experiments

The various special methods described were carried out very carefully. The result was that in almost every instance cultures from curly top tissue in all the various media described, and especially those which contained glucose, showed a heavy growth after twelve hours of incubation at 20°C. This result was practically uniform wherever diseased material had been used. Occasionally growth also appeared in cultures from supposedly normal plants, but in by far the great majority of cases such cultures remained sterile. This seemed to indicate that the organism was not peculiarly difficult to isolate, judging from its abundant growth on such a wide variety of media. Nevertheless a painstaking work was undertaken in order to complete the thorough study which had been planned. The anaerobic cultures also proved to be invaded by the same organism. Here, however, the growth was less abundant and extremely slow. Several days elapsed before any colonies were visible. A great deal of effort was further spent on work with all kinds of media but always the same organism grew abundantly. Contaminations naturally occurred now and then but the fact was most decidedly apparent that the one species announced by Smith and Boncquet predominated in the tissues of curly top plants to the practical exclusion of all others. The special culture methods described above are given in some detail, inasmuch as they may contain suggestions of value in similar work. Having found that this organism grew so easily and abundantly upon ordinary media, the use of special preparations was abandoned in the attempts to isolate the organism from plant tissues and the work was carried on entirely with standard bouillon to which 5 per cent glucose had been added. The object of the glucose was to promote the growth of the characteristic zoogloeae of this organism, rendering its identification in the original tubes easy without plating. By occasional plating, as a check on the work, it was soon possible to identify this organism very accurately by microscopic examination of tubes which showed the characteristic ring formation at the surface of the liquid. The

TABLE 1

Results of bacterial isolation experiments by cultural methods

(Tubes incubated at 28°C. *Disease* means curly top)

MATERIAL	NUMBER OF TUBES	RESULT
Petioles of curly top beets	6	5 tubes developed *B. morulans* within two days
Petioles of normal beets	6	Tubes remained clear for a week, when they were discarded
Petioles of diseased beets	9	8 tubes developed *morulans*
A leaf showing curly top symptoms on half of blade and in corresponding half of petiole. Other side appeared normal. This material from affected half of petiole with black streaks	5	All developed *morulans*
Corresponding half of blade	3	2 tubes developed *morulans*
Normal-appearing half of petiole	6	5 tubes developed *morulans* two days later than those from blackened part
Normal-appearing side of blade	3	No development
Petiole of a diseased center leaf	4	3 with *morulans*, 1 doubtful
Petioles of good-sized leaves from 5 different normal-appearing beets	10	2 tubes from 1 leaf both with *morulans;* others all clear
Typically diseased leaf with very slight dark streaks in the petiole	6	All developed *morulans*
Badly affected petiole of same beet. Pieces cut out with flamed scalpel	4	All developed *morulans*
Petioles of 4 beets from insect-proof cage. No sign of disease	8	Tubes from 2 plants remained clear; those from other 2 became slightly cloudy after several days, but no *morulans*
Petioles of 2 slightly diseased leaves. Pieces cut out with flamed scalpel	13	All tubes apparently containing pure cultures of *morulans*
A yellowish aster leaf	2	Remained clear
An old yellowish beet leaf without curly top. Tissue still sound	4	Some fungous growth. No *morulans*
Leaves of a somewhat abnormal-appearing beet but not with curly top	6	Some growth, but no *morulans*
Healthy-appearing leaves of aster, chrysanthemum, dahlia, tomato, bean, lettuce and raddish	24	11 tubes with fungi and bacteria, remainder clear. No *morulans* found
Petiole of typically diseased leaf, no disinfection	6	Very abundant growth of *morulans* intermixed with other organisms

TABLE 1—*Continued*

MATERIAL	NUMBER OF TUBES	RESULT
Petiole of slightly diseased leaf	2	Both very abundant *morulans*
Blade of same between veins	2	Both remained clear
Typically diseased leaf; scraped out interior portions of petiole with flamed scalpel after cleaning off epidermis	4	3 tubes developed *morulans*. 1 doubtful
A young leaf visibly affected on one side and very slightly at the base of the other side. Tissue taken from the most diseased side at base	4	All developed *morulans*
Terminal portion of diseased side, less visibly affected	4	2 developed *morulans;* 2 clear
Slightly affected base of other side of same leaf	4	1 developed *morulans;* 3 clear
Not visibly affected terminal portion of last	4	All remained clear

NOTE.—The last four are from the leaf illustrated in Phytopathology **5**: 106. The most elaborate precautions were taken to secure perfect surface disinfection and avoid contamination. These tubes in which growth appeared were plated out and found to contain pure cultures of *morulans*. The leaf was perfectly sound, showing only a slight roughening of the veins on the affected portion.

appearance to the eye of this ring, supplemented by microscopic examination, finding it to be composed of the characteristic zoogloeae, supplemented by occasional plating, is amply sufficient to identify this organism. A number of typical examples of isolation experiments with sugar beets are shown in table 1.

Several hundred illustrations similar to those shown in table 1 might be given. The results varied somewhat with the perfection of technique

TABLE 2

Bacillus morulans upon sugar beet seed

MATERIAL	NUMBER OF TUBES	RESULT
Beet seed imported from Germany, 1 dropped into each bouillon tube, with no previous treatment	10	At least 7 developed an abundance of *morulans*, mixed with other organisms
Similar seed previously soaked for twenty minutes in mercuric chlorid and washed in sterilized water	10	All clear

and in individual cases, but a mass of evidence was collected to indicate that this organism exists regularly in the interior of the foliage of sugar beets where the visible symptoms of curly top occur and that it does not develop in cultures from normal foliage or even the normal-appearing portions of partially affected leaves; also that it does not occur in the interior of beet leaves which may be yellow or sickly from ordinary causes.

Cultures from seed. Many attempts similar to those shown in table 2 were made to isolate the organism from sugar beet seed. The uniform result was that almost every unsterilized beet seed dropped into a tube of bouillon developed a very luxuriant growth of *Bacillus morulans.*

TABLE 3

Bacillus morulans from soil

MATERIAL	NUMBER OF TUBES	RESULT
Pinches of soil from about the roots of a diseased beet	4	*Morulans* was abundant in several of the tubes
Pinches of soil from about the roots of a normal beet in insect-proof cage	4	Some *morulans* present in the mixed growth resulting

Cultures from soil. That the organism is present in some soils is indicated by the data presented in table 3. The work was rather crude but certainly *B. morulans* was abundant in the soils examined.

Cultures from unsterilized foliage. Cultures made from unsterilized leaves of the sugar beet (table 4) show that the organism is common as a saprophyte upon the leaves of the plant, but in all cases when leaves similar to these were thoroughly disinfected no growth was obtained.

TABLE 4

Cultures from unsterilized foliage

MATERIAL	NUMBER OF TUBES	RESULT
Leaves of normal beets	10	Several contained an abundance of *morulans*

Unsterilized leaves of many other plants were also tried but the resulting growth was so mixed that no safe conclusions could be drawn. The only certain developments of *morulans* occurred in tubes inoculated with pieces of chrysanthemum leaves.

Cultures from sugar beet leaves with types of disease other than curly top. The fact that bodies resembling bacteria have been seen with the microscope in sugar beet leaves not affected with curly top, but affected with

various types of morphological abnormality, has been referred to in our recent article in Phytopathology. These various foliage types have never been definitely classified or described nor has the extent of the occurrence of this organism in other tissues been learned. In this cultural study,

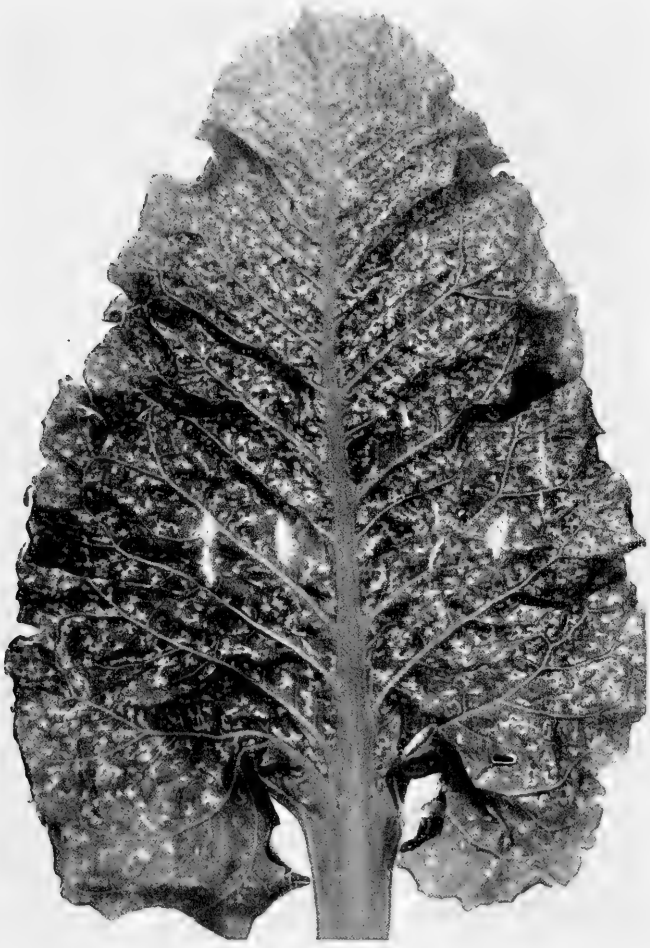

FIG. 4. MOTTLED LEAF OF SUGAR BEET

however, two at least of these forms gave the organism in abundance (table 5). In one of these, which we shall call mottled leaf (fig. 4), the blades of the leaves are strongly mottled with green and pale areas in a very characteristic manner. In the other type which we call black edge or black tip (fig. 5), the green leaves begin to die and turn decidedly black

about the margin or at the tips, with a yellow band of tissue between the black and the normal green. In some such leaves the development is very much suppressed, even to a point where the leaf consists of scarcely more than a petiole with a small blackened tip.

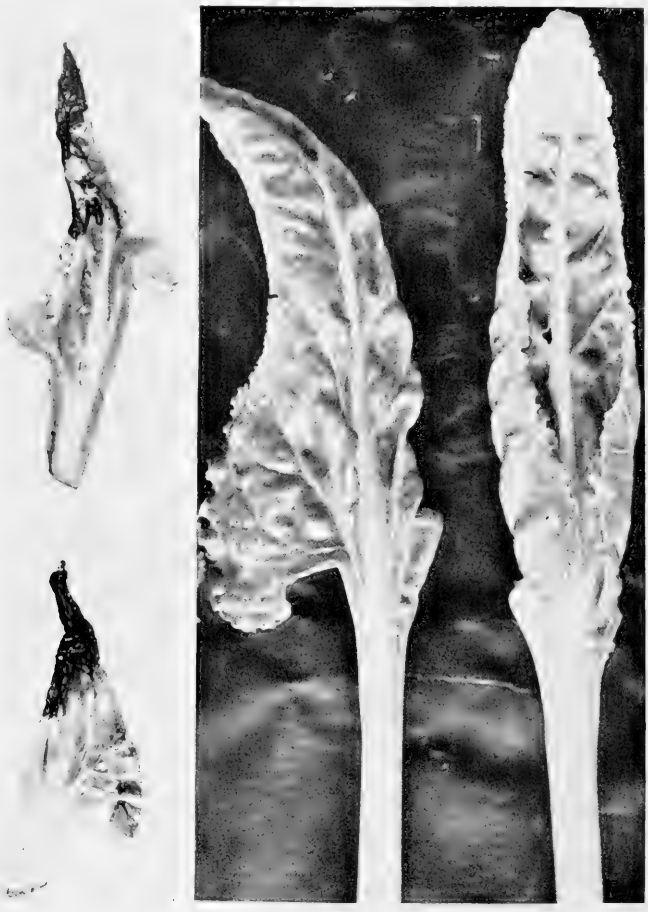

FIG. 5. BLACK EDGE AND BLACK TIP OF SUGAR BEET LEAVES
Black tip (left) and black edge of sugar beet leaves

Relation of mottled leaf and black edge to curly top. For some time it was believed that these conditions represented types or stages of curly top. Later, however, it was found that they occurred under circumstances which made this seem practically impossible. Further, when the experiment was tried of placing *Eutettix tenella* upon such plants the typical disease developed promptly.

TABLE 5

Cultures from sugar beet leaves with types of disease other than curly top

MATERIAL	NUMBER OF TUBES	RESULT
Yellowish area of young "black edge" leaf taken from between black and green portions. Thoroughly disinfected	2	Both gave a strong growth of *morulans*
Similar to last, but not disinfected	4	Very vigorous and apparently nearly pure growth of *morulans*
Similar material, disinfected	4	All with *morulans*
Similar material, disinfected	4	All with *morulans*
Typical "mottled leaf;" not disinfected. Blade, petiole and veins	9	All produced *morulans* in abundance
Petiole of "black edge" leaf, disinfected	4	All appear to have pure cultures of *morulans*
Petioles of normal appearing leaves from healthy plant. Very carefully disinfected	5	All tubes clear
Petiole of decidedly "mottled leaf." Inner tissue removed with flamed scalpel	4	Very vigorous growth of *morulans*

Further study of the organism, which was uniformly present in diseased beets

Although the organism was able to grow most abundantly on the common culture media, the peculiarity of this growth under all circumstances was of such a nature that for some time a continual contamination was suspected. Each separate colony seemed always to have two kinds of bacteria, very distinct in form. Very active bacteria were always observed at the edge of the colonies, while capsultated bacilli were generally observed in the middle. Therefore, before any further study of the organism was taken up, repeated efforts were made to separate these two widely distinct forms. For this purpose, the calcium carbonate and the India ink method for separating the individual organisms previous to plating them were resorted to.

Calcium carbonate. To 10 grams of calcium carbonate enough water was added to form a milky paste. This was subsequently introduced into a 200 cc. Erlenmeyer flask and sterilized in the autoclave. After the necessary cooling several young colonies of the bacteria were introduced into the semi-liquid mass and shaken for two hours so as to separate each individual organism from the other. From this paste, several plates were poured in the usual manner. They were incubated at 37°C. and closely examined as soon as any sign of development occurred.

India ink method. For this purpose special Chinese ink, prepared by Grübler, (*Punkttusche*) was used. A 15 per cent nutrient gelatin was made and poured into clay-covered petri dishes. Special care was given to prevent condensation water from flooding the medium. The Grübler's ink was diluted twenty times with n/6 glucose solution and sterilized in the autoclave. In a sterilized, empty petri dish ten drops of the ink were put in a row. The first drop was inoculated with a small amount of bacteria from a twelve-hours-old streak culture. The bacteria were thoroughly mixed with the ink of the first drop. Then a loop of this was transferred to the second drop and also thoroughly mixed. This transfer was repeated in the same way with the remaining drops in the dish. From the tenth drop, with a sterilized drawing pen, a small amount was taken. Small dots were made with the pen on a gelatin plate in such a way that the surface pellicule of gelatin remained uninjured. These ink dots were left to dry for two minutes then covered with a sterilized cover-glass. A small drop of immersion oil was subsequently applied to the cover-glass and the whole petri dish was brought to the microscope for examination. Each black point was then examined with microscope until one was found which contained one single organism. The organism appeared as a translucent dot on a black field. Its development was closely followed; the first division was distinctly noticed after half an hour; it multiplied rapidly; all the individuals were motile; they liquefied the gelatin slightly and moved about very briskly in the liquid under the cover-glass. After six hours some of the organisms became sluggish and gradually lost their motion. They increased in size and formed a capsule. Repeatedly they divided in the same capsule, stretching the jelly-like membrane more and more. The newly formed organisms within the original capsule also encapsulated in their turn (figs. 6 and 7). At the same time the individuals on the rim of the colony multiplied and remained motile. The double form of the bacillus was in this way clearly explained and proved.

IDENTITY OF THE ORGANISM FOUND IN CURLY TOP BEETS

A study of the literature of the subject shows that the greatest similarity to our organism of any described species is presented by that described by Arthur and Bolley[5] as *Bacterium Dianthi* as the cause of a leaf spot of the carnation. In its morphology, so far as described by these writers, this organism is very similar to ours, the resemblance being made pronounced by the development of characteristic zoogloeae. In biological behavior, however, the two organisms cannot be accurately compared, since the

[5] Arthur, J. C. and Bolley, H. L. Bacteriosis of carnations. Indiana Agr. Exp. Sta. Bul. 59. 1896.

work of Arthur and Bolley was carried on at a time when bacteriological technique was not standardized upon modern lines. One noticeable difference exists in respect to growth upon an acid medium, *B. Dianthi* being said to grow best under such circumstances, which is not the case with our organism. The description of the bacterial organism given by Arthur and Golden[6] and again by Miss Cunningham[7] as the cause of the so-called Indiana sugar beet disease, is similarly subject to uncertainty, but if this work was accurately done the organism must certainly have been different from ours in that it is said to be a particularly active gas former, which feature is totally lacking in our organism. It seems proper to mention here, however, the fact that Professor Arthur in a recent personal letter states that the accuracy of all this early work performed under his direction is open to some doubt on account of the undeveloped condition of bacteriological technique at the time and he expresses the opinion that the organisms found by Bolley, Miss Golden and Miss Cunningham were very likely identical.

The organism described in the unpublished work of Schneider[8] as *Bacillus californiensis*, which was isolated from curly top beets in California, seems again in its morphological characteristics to be entirely similar to ours and we feel little doubt that Schneider and the present writer had the same organism before them. Schneider found his organism also very abundant in sugar beet soils and upon the surface of the plants. He attributes a stimulative effect to this species, when applied in pure culture to sterilized beet seed or to the foliage of young plants.

The organism described by Düggeli[9] as being abundantly and often exclusively present upon the surface of various plants and seeds, seems also very similar to ours in form and size, formation of zoogloeae, color, saprophytic habitat and most biological characters. This was named by Düggeli *Bacterium herbicola aureum*, but "said to be the same as the *Bacillus mesentericus aureus*, isolated by Winkler from the surface of plum leaves." The latter statement confuses the identification.

DESCRIPTION OF THE ORGANISM

Summing up the whole situation, we feel justified in describing our organism as a new species on account of the incomplete and doubtfully

[6] Arthur, J. C. and Golden, K. E. Disease of the sugar beet root. Indiana Agr. Exp. Sta. Bul. 39, pt. 3: 54. 1892.

[7] Cunningham, C. A. A bacterial disease of the sugar beet. Bot. Gaz. **28:** 177–192. 1899.

[8] Schneider, A. The California beet blight. Spreckels Sugar Co. Exp. Sta. Rept. **23:** —. 1906. (Unpublished.)

[9] Centbl. Bakt. II, **12:** 602 and 695; **13:** 56 and 198. 1904.

accurate descriptions of those species which more or less resemble it, and the fact that none of them corresponds throughout.

Bacillus morulans n. sp.

Morphology

Vegetative cells. Grown in Liebig bouillon for sixteen hours at about 20°C., oval to short rods, single or in pairs. Grown at 37°, short rods in pairs or in short chains.

Size. Length 1.5 μ; breadth 0.9 μ; extreme length from 1.5 to 2 μ.

Capsules. Easily observed in 1/1000 glycerin bouillon after twenty-four hours and also in milk media (figs. 6 and 7).

Motility. Very active on agar and in bouillon, when grown at 37° for twelve hours.

Flagella. Stained by Zettnow's method; four long peritrichial flagella (figs. 6 and 7).

Pleomorphism. Cocciforms observed in glucose bouillon tubes and blood serum media after thirty days.

Stain. Easily with watery fuchsin, decolorized by Gram's method.

Cultural features

Gelatin plate neutral to phenolphthalein. Form, round to irregular; surface elevation, flat to convex contoured; internal structure, refraction strong, hyaline, moruloid; zoogloeae very marked; edges, entire to undulate; optical characteristics transparent to butyrous; consistency, viscous. Each colony is surrounded with many secondary colonies, appearing as small, oily drops of high refractive power. The appearance of zoogloeae is very noticeable in the middle of the colonies.

Gelatin plates 1.5 acid to phenolphthalein. The entire mass is a zoogloea, lobed and irregular in outline; the colony is slightly colored; orange-yellow, no surrounding colonies noticed.

Gelatin streak. After five days: growth, linear; margin, continuous; surface relief, flat to convex; light transmission, butyrous; color, yellow-orange; luster, glistening; consistency, viscous. The water of condensation has a yellow sediment.

Gelatin stab. After twenty-two hours, top growth: size, 5 mm., irregular contoured; pulvinate to capitate, light orange in color; viscous in consistency, luster shining.

Gelatin streak. Filiform to slightly beaded. After fifteen days, liquefaction of medium; crateriform with a yellow sediment. After twenty days, liquefaction stratiform; yellow pigment, decreased by absence of oxygen.

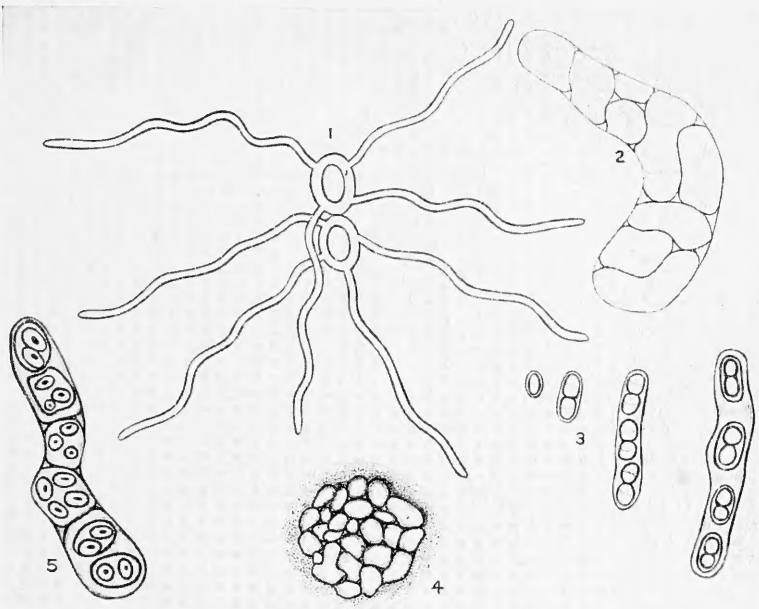

FIG. 6. MORPHOLOGY OF BACILLUS MORULANS

1, A chain of two individuals, showing flagella.

2, *3*, *5*, development of capsule and zoogloea from one individual. *4*, mass of zoogloeae.

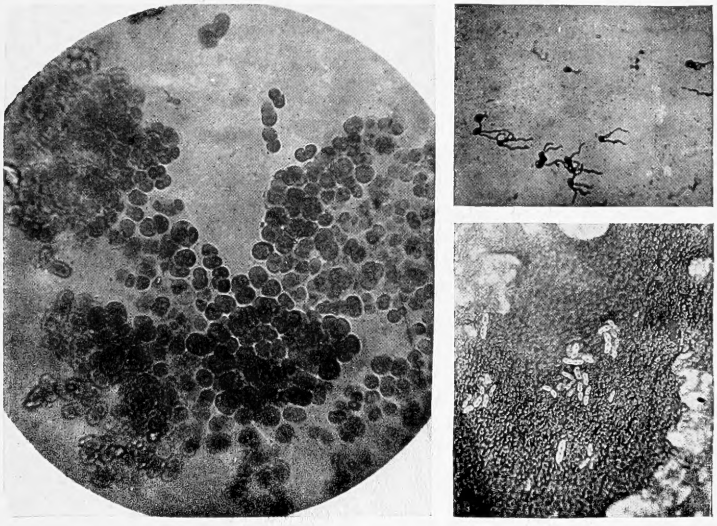

FIG. 7. BACILLUS MORULANS, SHOWING CAPSULES AND FLAGELLA

Agar. The colonies are extremely variable according to the density of growth, the moisture and the temperature.

Milk. Peptonisation of casein in fifteen days at 37°. The reaction is alkaline to Azolitmin.

Litmus whey. Remains clear, alkaline reaction.

Bouillon tubes. Opacity begins after eight hours at 37°, a pellicule forms in twenty-four hours or less. The color of the pellicule and the ring is dull, soft gray; thick, viscous and consists of conglomerate zoogloeae. These are generally oval, but may be linear and all united in chains.

Deposit, forms after two days incubation at 37°. Deposit is in the beginning slight, and finally yellow. The amount of deposit and the intensity of the color increases, however, rapidly. After ten days, a decidedly deep yellow-orange has developed. The deposit is compact and viscid on agitation.

Potato streak. After twenty four hours at 37°. Size, 2 mm.; sharp, linear; margin, continuous; color, yellow, homochromous; luster, glistening; texture, homogeneous. No liquefaction of potato and no gas formation.

Physical and biochemical features

Reaction. In carbohydrate-free media the reaction is alkaline; in carbohydrate media, the reaction is acid, except in lactose, where the reaction is slightly alkaline. See table 6.

Nitrate Liebig broth. After twenty-four hours at 37°, strongly reduced to nitrite.

Indol. Not produced in peptone solution after ten days.

Optimum temperature. 37°, measured by the amount of acid produced in 1 per cent glucose after five days. Acidity was 2.5.

Thermal deathpoint. Six-hours culture in bouillon; 54°C. in ten minutes.

Carbohydrate fermentation. Shown in table 6.

Resistance to mercuric chlorid. Six-hours culture on bouillon agar streak killed in 1/25,000 to 1/30,000 in ten minutes.

Relative growth in acid and alkalin media. Determined by the appearance of cloudiness in the tubes. Grows best on neutral or slightly alkalin media. Five per cent in acid apparently stops all growth; 7 per cent in alkaline: same.

Gas production. No gas is produced. See table 6.

Relation to free oxygen. Aerobic; facultative anaerobic.

TABLE 6

Carbohydrate fermentation of Bacillus morulans

(Incubation: 37°C., medium neutral to azolitmin)

MEDIUM: 1 PER CENT PEPTONE AND 1 PER CENT CARBOHYDRATE	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 5 DAYS	ACIDITY AFTER 5 DAYS	GAS AFTER 5 DAYS	DEPOSIT	FILM	RING	REACTION	
											Closed arm	Open arm
						$\times 10^{-5}$						
Dextrin	0	0	AB*	AB*	AB*		0	p	a	a	acid	alk.
Inulin	0	0	0	0	0	0	0	a	a	x	0	0
Amygdalin	0	0	0	0	0	0	0	a	a	a	0	0
Salicin	A	A	A	A	A	1.8	0	p	a	a	acid	acid
Glycerin	0	0	0	A	A	0.1	0	a	a	b	acid	acid
Lactose	0	0	B	B	B	0.2	0	a	a	a	basic	basic
Laevulose	A	A	A	A	A	2.3	0	b	b	p	acid	acid
Galactose	A	A	A	A	A	2.1	0	b	b	p	acid	acid
Dextrose	A	A	A	A	A	2.5	0	b	b	b	acid	acid
Saccharose	A	A	A	A	A	1.6	0	p	b	p	acid	acid
Mannit	A	A	A	A	A	1.3	0	p	a	x	acid	acid
Maltose	A	A	A	A	A	1.3	0	p	a	p	acid	acid
Rhamnose	A	A	A	A	A	1.3	0	p	a	x	acid	acid

NOTE.—*A*, acid; *B*, basic; *p*, permanent; *b*, abundantly present; *a*, absent; *x*, more or less present; *, acid on top and basic in tube.

Pathogenesis

One loop from a twelve-hours-old streak culture on bouillon agar introduced intravenously in a rabbit, caused death within twenty-four hours.

On *Dianthus incarnata.* The young unfolded leaves, when unrolled and covered with an abundant suspension of bacteria, developed small necrotic regions. The necrotic regions are watery and translucent on the edges, slightly elongate or irregular in outline, following the venation. The inside of the necrotic regions is slightly brown.

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